

THE PRESENCE OF FERTILIZIN HAPTENS WITHIN THE UNFERTILIZED SEA URCHIN EGG¹

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Tyler and Brookbank (1956a) have shown that rabbit antisera against purified fertilizin are capable of reacting with the hyaline layer of fertilized eggs, and of inhibiting the mitotic division of these eggs. This indicates a similarity between the hyaline layer combining groups or haptens and fertilizin haptens. Absorption of anti-fertilizin sera with sperm or coelomic fluid ("blood") does not remove the reaction of anti-fertilizin sera with fertilizin or hyaline layer material (Tyler and Brookbank, 1956b), indicating that species antigens are not involved. In addition, antisera against extracts of jelly-free unfertilized and fertilized eggs also possess properties of antisera against fertilizin (Tyler and Brookbank, 1956a). The possibility therefore exists that fertilizin haptens may be present within the eggs. The present report is concerned with the presence of fertilizin haptens within a granular fraction of the unfertilized egg.

MATERIALS AND METHODS

Fertilizins of *Strongylocentrotus purpuratus* (Friday Harbor, Washington) and *Lytechinus variegatus* (Sea Horse Key, Florida) were prepared from acid- (pH 3.5) treated unfertilized eggs by the method of Tyler (1949). After a pre-injection control bleeding, rabbits were injected on alternate days, over a three-week period, with ca. 50 μ g of fertilizin. Two intravenous injections alternated with a single intraperitoneal injection. The sera were recovered 4-5 days after the final injection, and thoroughly dialyzed against sea water. Reaction of the sera with fertilized eggs (cleavage block), fertilizin (ring precipitin test), and sperm (agglutination) was recorded.

Preparations of adenosine-triphosphatase-bearing granules (ATPase-granules) were made according to a method devised by Whiteley (unpublished data). Unfertilized eggs were deprived of soluble fertilizin by acid (pH 3.5) treatment, and thoroughly washed. Since acid-treated eggs are fertilizable, and are agglutinated by solutions of antifertilizin, it is apparent that some fertilizin remains on the surface after acid treatment. In order to reduce possible contamination of the ATPase-granules with this remaining fertilizin, the eggs were treated for 20

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minutes with 10 mg.% trypsin in sea water (crystalline, lyophilized trypsin, Worthington Biochemical Company, Freehold, New Jersey) prior to homogenization. Eggs so treated were found to have reduced (no greater than 25% cleavage in 0.25% sperm suspension) fertilizability as compared with controls (ca. 90% cleavage in 0.25% sperm suspension), and also a reduced capacity to absorb antibodies against fertilizin (Table I). In order to minimize the amount of fertilizin present on the surface, the eggs were, therefore, routinely trypsinized in this manner prior to homogenization. One ml. of the washed, settled eggs was homogenized with 9 ml. of cold KCl-citrate solution (1 part .35 *M* Na citrate: 9 parts .55 *M* KCl, pH 6.8). Following two low speed centrifugations to remove unbroken eggs etc., the ATPase-granules were recovered, as a yellow pellet, by two successive centrifugations of 15 minutes duration (10° C.) at 10,000 × gravity. The re-suspended particles were spherical, of uniform size (ca. 2 microns in diameter) and readily distinguishable from the larger yolk granules which remain, for the most part, in the supernatant. Such small-granule preparations have 85% of the total ATPase activity of the whole egg homogenate (Whiteley, unpublished data). Prep-

TABLE I

The effect of trypsin treatment on the capacity of unfertilized eggs to absorb antibodies against fertilizin

Serum		Ring with fertilizin	
# 10	(pre-injection serum)	±	
10'	(antiserum) unabsorbed	+++	
10'	trypsinized egg absorbed	+++	2nd absorption +
10'	egg absorbed (no trypsin)	+	2nd absorption ±

arations of this sort, with no more than an estimated 10% contamination by yolk granules, were used as absorbing antigens (preparations which contained little or no discernible yolk were also successfully utilized). Prior to use in absorptions, the yellow pellets were rinsed with sea water to remove the KCl-citrate mixture.

Sperm which were used as absorbing antigens were centrifuged and washed three times to remove seminal fluid. "Blood" was obtained from KCl-injected adult animals through a puncture in the peristomial membrane, and examined for contamination with eggs or sperm. This material was then allowed to clot at room temperature. The clot was recovered by centrifugation, washed with sea water, and used as an absorbing antigen. Jelly-free unfertilized eggs, deprived of the soluble portion of their fertilizin coat by acid treatment (pH 3.5), were also used for absorptions. For each experiment, approximately equal volumes of serum and absorbing material were mixed for 5 minutes at room temperature, and then centrifuged to recover the serum.

Clear supernatants of homogenized blood clots and homogenized ATPase-granule supernatant were used as test antigens in ring precipitin tests, as were fertilizin solutions. Visible precipitin reactions occurring within 2-5 minutes were scored as +++, while those appearing after 30 minutes are indicated as +. Reactions appearing after 90 minutes are indicated by ±. Tests were carried out with undiluted sera in tubes of ca. 1.5 mm. internal diameter.

Tests for cleavage blocking activity involved mixing one drop of egg suspension (about 100 eggs) and one drop undiluted serum (sea water dialyzed). The sera

were scored as blocking (+++) if no more than one cell division of the membraneless fertilized eggs occurred following the addition of the serum. Retardations of development without inhibition of cleavage are indicated by a plus-minus sign (\pm).

RESULTS AND DISCUSSION

The results of experiments with the two species of sea urchin are summarized in Table II. From this table it can be concluded that absorption with eggs or ATPase-granules removes most of the cleavage-blocking activity of the immune sera, as well as the majority of the fertilizin precipitins; sperm absorption is not effective, nor is absorption with blood. Absorption with blood removes virtually all antibodies against this material, though sperm absorption leaves antibodies, capable of precipitating soluble blood antigens, in solution. Since the unabsorbed *Lytechinus* antisera against fertilizin do not react with sperm, it is reasonable that sperm absorption should fail to abolish any reactions exhibited by these sera. The reaction of *L. variegatus* anti-fertilizin sera with ATPase-granule supernatant may be due to the presence of unsedimented ATPase-granules in this material, or to the presence of fertilizin haptens associated with some other cytoplasmic fraction.

Concerning contamination of the ATPase-granules with surface fertilizin, all that can be said, at present, is that attempts have been made to hold this to a minimum. As can be seen in Table I, the trypsinized eggs show only a *reduction* in ability to absorb antibodies against fertilizin, indicating that some fertilizin remains after this treatment (further trypsin treatment tends to make the eggs excessively fragile, and therefore difficult to handle). Quantitative studies on the amount of granular material necessary to absorb a given volume of antiserum are necessary before the effectiveness of trypsin treatment in reducing possible contamination of the ATPase-granules can be evaluated. On the other hand, the acid-treated eggs were washed three times with sea water, and, following trypsin treatment, an additional five times with sea water and three times with the homogenization medium. It is therefore unlikely that any soluble fertilizin is available, for adsorption to internal constituents, at the onset of homogenization. Small particles of fertilizin, in an insoluble complex of some sort, may be present and able to combine with ATPase-granules or other fractions.

These results are of value in interpreting the similarity of action of antisera against purified fertilizin and antisera against extracts of "jelly-free" unfertilized and fertilized eggs. Both types of antisera block cleavage, precipitate fertilizin, and may fail to agglutinate sperm (Tyler and Brookbank, 1956a, 1956b). In addition, antisera against *Lytechinus* fertilizin, and against "jelly-free" unfertilized and fertilized *Lytechinus* eggs, increase the respiration rate of unfertilized and fertilized eggs (Tyler and Brookbank 1956b; Brookbank, 1959).

The presence of fertilizin haptens within the eggs may be of importance in the chemical "architecture" of the eggs. Tyler (1940) discovered that unfertilized sea urchin eggs contain a substance complementary to the surface fertilizin (termed antifertilizin from eggs). This discovery and other investigations (Tyler, 1946) led Tyler (1947) to propose an auto-antibody concept of cell structure and cell adhesion, involving a system of interlocking, mutually complementary substances extending from sites of synthesis to the external boundary of the cell. The demonstration of fertilizin haptens within the eggs would be consistent with this

TABLE II

*Ring tests, sperm agglutination, and cleavage inhibition
tests on antisera against fertilizin*

	Ring with fertilizin	Agglutina- tion of sperm	Cleavage block	Ring with "blood"	Ring with ATPase granule supernatant
<i>S. purpuratus</i>					
Pre-injection sera					
d. unabsorbed	o	o	o		
d. sperm absorbed	o	o	o		
d. ATPase-granule absorbed	o	o	o		
e. unabsorbed	±	o	o		
e. sperm absorbed	±	o	o		
f. unabsorbed	o	o	o		
f. sperm absorbed	o	o	o		
Immune sera					
d. unabsorbed	+++	+	+++		
d. sperm absorbed	+++	o	+++		
d. ATPase-granule absorbed	o	+	±		
e. unabsorbed	+++	o	+++		
e. sperm absorbed	+++	o	+++		
f. unabsorbed	+++	±	+++		
f. sperm absorbed	+++	o	+++		
<i>L. variegatus</i>					
Pre-injection sera					
#4. unabsorbed	±	o	o	o	o
#4. sperm absorbed	±	o	o	—	—
#4. blood absorbed	o	o	—	o	—
#4. unfert. egg absorbed	±	—	o	—	o
#4. ATPase-granule absorbed	o	—	o	—	o
#10. unabsorbed	±	o	o	o	—
#10. sperm absorbed	±	o	o	o	—
#10. unfert. egg absorbed	o	—	o	—	—
#10. ATPase-granule absorbed	±	—	o	—	—
Immune sera					
4. unabsorbed	+++	o	+++	+	+
4. sperm absorbed	+++	o	+++	+	—
4. blood absorbed	+++	o	+++	±	—
4. unfert. egg absorbed	±	—	o	—	±
4. ATPase-granule absorbed	±	—	o	—	o
10. unabsorbed	+++	o	+++	+	—
10. sperm absorbed	+++	o	+++	+	—
10. unfert. egg absorbed	±	—	o	—	—
10. ATPase-granule absorbed	+	o	±	—	—

— indicates test not performed.

theory. However, the presence of fertilizin haptens, as demonstrated by serological techniques, does not, of course, necessarily imply that the substances bearing these haptens are able to combine specifically with antifertilizin of eggs of sperm. Or, to rephrase the foregoing sentence, there is no reason to assume that the rabbit

antibodies against fertilizin are directed, exclusively or in part, against the fertilizin-antifertilizin combining sites.

SUMMARY

1. ATPase-bearing granules of unfertilized sea urchin eggs were shown, through absorptions of antisera against fertilizin, to possess fertilizin-like combining groups.

2. These granules were also capable of neutralizing the cleavage-blocking action of these antisera.

3. These results are discussed in light of the similarity of action of antisera against purified fertilizin to the action of antisera against extracts of jelly-free (acid treated) unfertilized eggs, and washed, demembranated fertilized eggs.

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